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(54) Title: THERAPEUTIC COMPOSITIONS

(57) Abstract: The present invention relates to a composition comprising a boron-containing compound and a polyhydroxy acid in a solution having a pH of 7.5 to 10.5. This composition provides a boron-containing solution at a therapeutically usable level. There is further provided a composition comprising a stable particulate suspension of a boron-containing compound and a surfactant. The boron-containing composition of the present invention are provided for use in the treatment of cancer specifically in boron neutron capture therapy, radiotherapy, or other ionising or non-ionising photon or atomic or sub-atomic particle therapy.

WO 2004/030661 A2

Therapeutic Compositions

The present invention relates to a composition comprising a boron containing compound and a polyhydroxy acid in a solution having a pH of 7.5 to 10.5 and to a process for producing such a composition. The invention further provides a composition comprising a stable particulate suspension of a boron containing compound and a surfactant. The invention also provides the above compositions for use in medicine, particularly for use in the treatment of cancer.

Conventional anti-cancer therapies such as radiotherapy have a number of disadvantages. In radiotherapy the biological effect of the therapy is spread over the entire irradiated area, and a high radiation dose is required to generate the required destructive ionisation tracks.

Boron neutron capture therapy (BNCT) in contrast involves the preferential accumulation of boron in the tumour rather than healthy tissue. Targeting of the boron isotope using high energy short range particles allows *in situ* selectivity. This targeting allows the production of a relatively large radiation dose in the tumour compared with the surrounding healthy tissue.

The use of high energy, short range particles demonstrate a greater destructive potential than the conventional low energy radiation beams used. As a result, lower doses of neutron capture therapy are required to produce the same therapeutic effect as conventional therapy. In addition, because neutron capture therapy is not dependent upon the oxygen levels of the targeted cells, it can provide effective therapy where the tumour is anatomically compromised and therefore hypoxic.

Boron neutron capture therapy can be used to treat a variety of cancers which are normally treated with radiotherapy, including for example lymphomas and skin cancers, as well as cancers of the breast, lung, head and neck, bone, prostate, pancreas and cervix. Boron neutron capture therapy may also be used in combination with surgery to help shrink the size of the tumour and reduce normal tissue loss. In contrast to conventional radiotherapy, which is administered up to 30 times in a six week period, boron neutron capture therapy can be administered over a period of 2 to 4 days, and as a result is less demanding for the patient.

Sources of boron used in boron neutron capture therapy include the boron-containing amino acids, such as boronophenylalanine (BPA) and boron carriers including carborane cages (Solway *et al*, Chem. Rev., 1998, 98, 1515-1562) halogenated sulfidohydroboranes (US Patent Nos. 5,455,022, 5,612,017 and 5,653,957, the contents of which are incorporated herein by reference), porphyrins (US Patent No 4,959,356, the contents of which are incorporated herein by reference), boronated porphyrins (US Patent No 5,877,165, the contents of which are incorporated herein by reference), small polymer chains, and dendromers and complex polymers such as starburst molecules (Soloway *et al*, the contents of which are incorporated herein by reference).

While sources of boron such as boronophenylalanine are effective in the treatment of various tumours such as malignant melanoma and glioblastoma with boron neutron capture therapy, the poor solubility of these boron-containing compounds can lead to problems with their administration.

BPA, for example, is only very sparingly water soluble (its water solubility is less than 1-2mg /ml). The poor water solubility of BPA has been one of the

major limiting factors in the advancement of BPA – boron neutron capture therapy treatment.

Previously BPA has been administered as an oral preparation in man in the form of a crude water based slurry of 800mg-1200mg/kg. However, in excess of 99.9% of the compound fails to enter the systemic circulation and is not available for tumour uptake. This makes the delivery of BPA very bio-inefficient and prohibitively expensive since a vast amount of the BPA fails to reach the target tumour mass. The small amounts of BPA that enter into the circulation have a short half-life of less than 60 minutes since it is rapidly excreted by the kidneys. The target tumour tissue fails to experience sufficiently high levels of compound for any significant period of time so as to accumulate a therapeutically effective concentration of BPA for effective boron neutron capture therapy.

To overcome the short blood half-life of the compound, conventionally successive multiple doses are given to gradually build up the concentration of BPA in the target tissue. For BNCT to be successful, concentrations in excess of 20μg/gm of boron in the target tissue, are required together with a maximum tumour to blood differential. While a typical ratio of 2.2: 1 of tumour:blood can be obtained using multiple dosing, higher ratios would be desirable in order to provide a more effective concentration of boron at the target tumour.

In practise, intravenous administration via continuous infusion has been favoured over the oral route of administration. However, this has lead to toxicity in other organs exposed to the drug, primarily in the kidney.

US 5935944 discloses the use of a 30mg/ml boronophenylalanine in a fructose solution. This solution is used as an intravenous administration to deliver a

dose of 600mg-1200mg /kg as a slow continuous infusion over a period of 2 hours. A total of 3-4 doses are required over a period of 8-12 hours. However, for this regime a total of over 280 g of BPA per 70 kg patient is required with a total fluid volume of 5.6 - 11.2 litres. The administration of such a high volume of fluid to already seriously ill patients is considered to be clinically unacceptable. The administration of high volumes of fluids causes haemodynamic complications that can lead to cardiac arrest and kidney malfunction. Therefore, the current 30mg/ml fructose based BPA-fructose intra venous formulation is restricted to experimental use and is not a clinically acceptable system for wide spread routine treatment.

Administration of boron in the form of boronated porphyrins such as CuTCPH and CuTCPBr are usually administered parenthally. CuTCPH is usually administered in a formulation comprising a 9% Cremophor EL, 20% propolyene glycol in Saline. 1.5% THF is used to solubilise CuTCPH. However, Cremophor EL has been associated with anaphylatic reactions and erythrocyte aggregation and this formulation is therefore unsuitable for clinical use.

CuTCPH and CuTCPBr both show low solubilities. Previous work has been carried out using these boronated compounds at concentrations of only 3.5mg/ml to 5mg/ml. The low water solubility of boron has therefore made the provision of high concentrations of boron containing compounds difficult. In addition, large amounts of the boronated compounds (over 70-90%) is lost in the liver and spleen after administration allowing only 1-2% to reach the tumour. The low solubility of the boronated compounds coupled with the poor bioavaliability means that a dose of 100-300mg/kg body weight is required to deliver sufficient boronated compound to the tumour mass. The low delivery of the boronated compounds (less than 2%) to the tumour and the unwanted

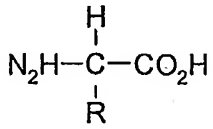
distribution of the boronated compounds to the other organs such as the liver, spleen, skin and lung is a major disadvantage of these formulations.

There is therefore a need for the provision of boron for boron nuclear capture therapy at higher and clinically useful levels.

The first aspect of the present invention relates to a composition comprising a boron containing compound and a polyhydroxy acid in a solution having a pH of from approximately 7.5 to 10.5, preferably approximately 8 to 10, more preferably approximately 8.0 to 9.1.

The solution of the present invention is preferably aqueous but may contain an organic solvent such as ethanol, propan-2-ol or polyethylene glycol 300. Preferably the organic solvent is water miscible and is present at a level of up to 50%, preferably 40% or less, more preferably 20% or less, most preferably 10% or less.

The boron containing compound can be boron or a boron-containing cluster having from 1 to 30 boron atoms, preferably 2 to 20 boron atoms, more preferably 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 boron atoms most preferably having 4, 5, 6, 10, 12 or 19 boron atoms such as $(B_{10}H_{13})_2$, $(B_{10}H_{13})_2O$, $PhCB_9H_9$, $(CO_5)CO_2B_{10}H_8(SMe_2)_2$, or a compound of formula I



(I)

wherein R is a group comprising one or more boron atoms.

The boron containing compound is preferably a compound of formula I wherein R is boron or a group containing one or more boron atoms or a boron cage such as dicarborane $C_2B_{10}H_{11}$ or a boron cluster most preferably having 4, 5, 6, 10, 12 or 19 boron atoms such as $(B_{10}H_{13})_2$, $(B_{10}H_{13})_2O$, $PhCB_9H_9$, $(CO_5)CO_2B_{10}H_8(SMe_2)_2$.

The boron containing compound is preferably boronophenylalanine. Other alternative boron sources for the purpose of this invention include para-boronophenyalanine, (D and L isomers), meta-boronophenyalanine, (D and L isomers), ortho-boronophenyalanine, (D and L isomers), boronophenylalanine analogues derivatised at the carboxy functionality i.e. mono-, di- and tetrahydroxy amides of boronophenylalanine, ^{18}F-Boronophenyalanine (para, meta and ortho versions) (D and L isomers), ammoniacarboxyborane, o-carboranylalanine (L and D isomers), boron containing analogues of methionine, DL-S(10-dimethylsulfidooctahydrodecarboranyl)methionine, BSN-glutathione disulfidine derivatives, naturally abundant ^{11}B or the ^{10}B isotope of boron or carborane cage containing amino acids. Preferably, boron is provided as the ^{10}B isotope.

Without being bound by scientific theory, it is believed that the presence of the polyhydroxy acid results in a clear, stable solution of the complexed boron containing compound (e.g. BPA) at high concentrations. The first aspect of the invention therefore provides a composition wherein the boron containing compound is provided at high concentrations. The composition of the present invention preferably provides concentrations of the boron containing compound e.g. boronophenylalanine in aqueous solution of 50mg/ml to 300mg/ml, preferably 100-300mg/ml, more preferably 200-250mg/ml.

These high concentrations of soluble boron lead to higher infusion concentrations of the active drug, providing higher concentrations of boron in the target tissue and thereby allowing an improved ratio of boron in the tumour tissue compared with the surrounding tissue. In addition, the provision of more concentrated solutions of boron will allow a major reduction in the total fluid volume administered to a patient thereby eliminating or substantially reducing complications arising from potential imbalance of haemodynamics.

In addition, the composition of the first aspect is stable over time periods up to 24 months, preferably 12-18 months, more preferably 5 or more months, allowing the composition to be stored prior to administration with little or no deterioration in the quality of the composition. This stability allows the composition to be supplied in solution making the composition more time efficient and convenient. There is furthermore less wastage of the composition thereby making the composition more economical.

For the purposes of this invention, polyhydroxy acids are carboxylic acid derivatives of mono- and/or di-saccharides and include one or more of lactic acid, citric acid, glucaric acid, gluconic acid, lactobionic acid, erythronic acid, glycerophosphate, mannuronic acid, levulinic acid or sorbic acid. Polyhydroxy acids does not relate to mono-saccharides or saccharides per se such as fructose.

The polyhydroxy acid is preferably provided with the boron-containing compound at a molar ratio of from 1:10 to 10:1 (polyhydroxy acid: boron containing compound),. preferably 0.1 : 2.0 (polyhydroxy acid: boron containing compound), more preferably 0.75 : 1.25.

The composition of the first aspect of the invention may further comprise one or more keto substituted or polyhydroxy sacharrides such as fructose, saccharine or lactose. It has been found that the presence of such a saccharide in the composition leads to increased solubility and stability of the composition. The saccharide is preferably added at a molar ratio of 0.01 : 0.25 to the boron containing compound, preferably 0.1 : 1.0. The presence of the saccharide further extends the shelf life of the formulation of the invention and increases the solubility of the boron containing compound thereby allowing the formulation to be prepared with a lower pH (i.e. closer to neutral).

In a preferred feature of the invention, fructose is provided at a level of approximately 0.1 mole/mole of BPA in the presence of molar ratios of BPA/saccharide acid of 1 / 0.8-1.0. Such levels lead to a significantly increased solubility and stability of the formulation. In particular concentrations in excess of 200-300mg /ml can be generated that remain stable and clear without any precipitation.

The present invention further provides a composition of the first aspect optionally containing an emulsifier.

These emulsifiers are any which are known in the art and can be one or more well known organic and inorganic pharmaceutical excipients including various polymers, low molecular weight oligomers, natural products and preferably ionic and non-ionic surfactants.

The composition may comprise one or more emulsifiers selected from gelatin, casein, lecithin (phosphatides), gum acacia, calcium stearate, cholesterol, tragacanth, sorbitan esters, stearic acid, benzalkonium chloride, glyceryl monostearate, cetostearl alcohol, cetomacrogol emulsifying wax, ,

polyoxyethylene alkyl ether (e.g. macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (eg. Commercially available Tweens, polyethylene glycols, polyoxyethylene stearates, phosphates, sodium dodecylsulphate , bile acids such as deoxycholic acid , cholic acid chenodeoxycholic acid, tauric acid etc., as their ammonium or sodium salts triethanolamine, polyvinyl alcohol (PVA), and polyvinypyrrolidonr (PVP). (Handbook of Pharmaceutical Excipients by American Pharmaceutical Association and The Pharmaceutical society of Great Britain, Pharmaceutical Press 1998).

Particularly preferred emulsifiers include one or more of lecithin, Pluronic F-68, polyvinylpyrrolidone (Kollidin 12F, BASF), polyoxyethylene 20 sorbitan monolaurate, polyoxyethylene 20 sorbitan monopalmitate, polyoxyethylene 20 sorbitan monostearate and polyoxyethylene 20 sorbitan monooleate or mixtures thereof. The emulsifiers are provided at levels of approximately 0.01 to 50%, more preferably 1 to 10%.

For the purposes of this invention, the emulsifiers preferably exhibit "stealth capabilities. Such emulsifiers, as polymers or layers have a very low toxicity profile, do not elicit an immune response, are non-thrombogenic and do not effect liver enzymes. When these compounds are used as emulisifiers in drug formulations, the endoreticular system has difficulty in detecting the particle thereby reducing the amount of the drug captured by the spleen and liver. This results in an increase in the bioavaliability of the drug and low total dose requirements.

The preferred polymers which may assist with stealth characteristics include (i) block copolymers of ethylene oxide and propylene oxide; polyvinyl pyrrolidone, Pluronic F68 and F108, (ii) Tetronic 908, a tetrafunctional block

copolymer derived from the addition of ethylene oxide and propylene oxide to ethylenediamine, dextran, lecithin, Carbowax 3350 and polyethylene glycols, aerosol OT(dioctyl ester of sodium sulfosuccinic acid from American Cyanamide), Tween 80 (polyoxyethylene sorbitan fatty acid ester, ICI chemicals), Duponol P (sodium lauryl sulfate, Dupont), Triton X-200 (alkylaryl polyether sulfonate, Rohm and Haas).

The second aspect of the invention provides a process for the preparation of the composition of the first aspect. Such process comprises mixing a boron containing compound with a polyhydroxy acid and optionally with fructose or an emulsifier in an aqueous solution. The pH of the solution can be raised to from 7.5 to 11 by the use of a suitable base.

Preferably, the pH is raised to from 8 to 10, by the use of a suitable base such as ammonium hydroxide, sodium hydroxide or sodium carbonate, calcium hydroxide, mono-, di-, or tri- ethanolamine or other physicologically acceptable substituted amine. Preferably, the polyhydroxy acid is added in a molar ratio ranging from 1 : 10 to 10 : 1 in water.

The final pH may be adjusted to a more physiologically acceptable value by the use of dilute mineral acids. Alternatively, the pH of the solution may not be altered after the production of the formulation.

All preferred features of the first aspect of the invention also apply to the second aspect.

The third aspect of the invention provides a composition comprising a stable particulate suspension of a boron containing compound and a surfactant. Preferably, the boron containing compounds include one or more of CuTCPH,

CuTPBr, boronophenylalanine, para-boronophenyalanine, (D and L isomers), meta-boronophenyalanine, (D and L isomers), ortho-boronophenyalanine, (D and L isomers), boronophenylalanine analogues derivatised at the carboxy functionality i.e. mono-, di- and tetrahydroxy amides of boronophenylalanine, ^{18}F-Boronophenyalanine (para, meta and ortho versions) (D and L isomers), ammoniacarboxyborane, o-carboranylalanine (L and D isomers), boron containing analogues of methionine, DL-S(10-dimethylsulfidooctahydrodecarboranyl)methionine, BSN-glutathione disulfidine derivatives, natural or artifical boron or carborane cage containing amino acids. Preferably, the boron-containing compound is sparingly water soluble i.e. with solublity less than 10-50mg/ml, preferably less than 1-5 mg per ml, more preferably less than 0.1-1mg/ml.

The composition of the third aspect provides high concentrations of the boron containing compound in a stable suspension.

The composition of the third aspect comprises particles of the boron containing compounds coated with surfactant with a particle diameter of 1000nm or less, preferably 400nm or less, more preferably 200nm or less.

Without being bound by scientific theory, the surfactants are understood to produce biocompatable layers via adsorbtion onto the particulate core structure. The surfactants do not necessarily chemically react to the surface and do not necessarily have any intermolecular cross-linkage.

For the purposes of the present invention, the surfactants are selected from a group consisting of organic and inorganic pharmaceutical excipients including polymers, low molecular weight oligomers, natural products and ionic and non-ionic surfactants.

The composition of the third aspect comprises one or more surfactants selected from gelatin, casein, lectine (phosphatides), gum acacia, calcium stearate, cholesterol, tragacanth, sorbitan esters, stearic acid, benzalkonium chloride, glyceryl monostearate, cetostearl alcohol, cetomacrogol emulsifying wax, , polyoxyethylene alkyl ether (e.g. macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (eg. Commercially available Tweens, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, colloidal titanium dioxide, phosphates, sodium dodecylsulphae, caroxymethycellulose calcium or sodium, methylcellulose, hydroxyrthylcellulose, hydroxypropylmethycellulose phthalate, noncrystalline cellulose, hydroxypropylcellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), and polyvinypyrrolidone (PVP). (Handbook of Pharmaceutical Excipients by American Pharmaceutical Association and The Pharmaceutical society of Great Britain, Pharmaceutical Press 1998).

The emulsifiers preferably assist with stealth characteristics as defined for the second aspect of the invention. The preferred biocompatable stealth layers include (I) block copolymers of ethylene oxide and propylene oxide; polyvinyl pyrrolidone, Pluronic F68 and F108, (ii) Tetronic 908, a tetrafunctional block copolymer derived from the addition of ethylene oxide and propylene oxide to ethylenediamine, dextran, lecithin, Carbowax 3350, polyethylene glycols, aerosol OT(dioctyl ester of sodium sulfosuccinic acid from American Cyanamide), Tween 80 (polyoxyethylene sorbitan fatty acid ester, ICI chemicals), Duponol P (sodium lauryl sulfate, Dupont), Triton X-200 (alkly aryl polyeter sulfonate, Rohm and Hass).

Preferably, the surfactants are one or more of lecithin, Pluronic and F-68 polyvinylpyrrolidone. polyoxyethylene 20 sorbitan monolaurate, polyoxyethylene 20 sorbitan monopalmitate, polyoxyethylene 20 sorbitan monostearate and polyoxyethylene 20 sorbitan monooleate or a mixture thereof but are not limited thereto.

The surfactant can be added in amounts ranging from 0.01 to 50% wt/wt, preferably 0.1 to 2% wt/wt, more preferably 0.1% to 1% wt/wt.

The fourth aspect of the invention relates to a process for the production of the composition of the third aspect. This process involves grinding the boron containing compound to form a powder with an average particle size of approximately 1000nm or less, preferably 400nm or less, more preferably 200nm or less and contacting the particles with a surfactant in an aqueous solution which may optionally contain one or more organic solvents. The organic solvent is preferably water miscible such as ethanol propan-2-ol or polyethylene glycols. The boron containing compound can be ground at room temperature or up to 37°C. The grinding process utilises rotation speeds of 1-100Hz, preferably 1-50Hz, more preferably 1-10Hz.

The fifth aspect of the present invention comprises a formulation comprising a composition of the first aspect in combination with a composition of the third aspect. The composition of the first and third aspects may be administered sequentially, simultaneously or separately. For example, the compositions of the first and third aspects may be provided in a single injection or in two separate injections.

The sixth aspect of the present invention provides a process for the preparation of the formulation of the fifth aspect. Such process provides combining the

composition of the first aspect with the composition of the third aspect. The formulation of the sixth aspect can be provided as a mixture of the composition of the first aspect and the composition of the third aspect. Alternatively the composition of the first aspect and the composition of the third aspect can be admixed immediately prior to administration.

The seventh aspect of the invention relates to the composition of the first or third aspects or the formulation of the fifth aspect for use in medicine. In particular, the seventh aspect relates to the composition of the first or third aspects or the formulation of the fifth aspect for use in the treatment of cancer, specifically in boron neutron capture therapy, radiotherapy, or other ionising or non-ionising photon or atomic or sub-atomic particle therapies such as photon, carbon ion or proton therapies. For the purposes of the seventh aspect, the composition of the first or third aspects or the formulation of the fifth aspect is for use in the treatment of cancer of the breast, lung, head, neck, bone, prostate, pancreas, cervix, skin and lymphomas. The composition of the first or third aspect or the formulation of the fifth aspect can be used in radiotherapy.

The compositions and/or formulation according to the invention for use in the aforementioned indications may be administered by any convenient method, for example by oral (including by inhalation), parenteral, mucosal (e.g. buccal, sublingual, nasal), rectal or transdermal administration and the compositions adapted accordingly.

For oral administration, the compositions and/or formulation can be formulated as liquids or solids, for example solutions, syrups, suspensions or emulsions, tablets, capsules and lozenges.

A liquid formulation will generally consist of a suspension or solution of the compositions and/or formulation in a suitable aqueous or non-aqueous liquid carrier(s) for example water, ethanol, glycerine, polyethylene glycol or an oil. The formulation may also contain a suspending agent, preservative, flavouring or colouring agent.

A composition and/or formulation in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and microcrystalline cellulose.

A composition and/or formulation in the form of a capsule can be prepared using routine encapsulation procedures. For example, powders, granules or pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule.

Compositions and/or formulations for oral administration may be designed to protect the active ingredient against degradation as it passes through the alimentary tract, for example by an outer coating of the formulation on a tablet or capsule.

Typical parenteral compositions and/or formulations consist of a solution or suspension of the compound or physiologically acceptable salt in a sterile aqueous or non-aqueous carrier or parenterally acceptable oil, for example polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oil or sesame oil.

Alternatively, the solution can be lyophilised and then reconstituted with a suitable solvent just prior to administration.

Compositions and/or formulations for nasal or oral administration may conveniently be formulated as aerosols, drops, gels and powders. Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomising device. Alternatively the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal once the contents of the container have been exhausted. Where the dosage form comprises an aerosol dispenser, it will contain a pharmaceutically acceptable propellant. The aerosol dosage forms can also take the form of a pump-atomiser.

Compositions and/or formulations suitable for buccal or sublingual administration include tablets, lozenges and pastilles, wherein the active ingredient is formulated with a carrier such as sugar and acacia, tragacanth, or gelatin and glycerin.

Compositions and/or formulations for rectal or vaginal administration are conveniently in the form of suppositories (containing a conventional suppository base such as cocoa butter), pessaries, vaginal tabs, foams or enemas.

Compositions and/or formulations suitable for transdermal administration include ointments, gels, patches and injections including powder injections.

Conveniently the composition and/or formulation is in unit dose form such as a tablet, capsule or ampoule.

In a preferred feature of the seventh aspect, the compositions of the first and third aspects are administered simultaneously, separately or sequentially.

The present invention is not restricted to the treatment of cancer by BNCT, or radiotherapy or other ionising or non-ionising photon or atomic or sub-atomic particle therapies such as photon, carbon ion or proton therapies but may be used in any method which requires BNCT or radiotherapy or other ionising or non-ionising photon or atomic or sub-atomic particle therapies such as photon, carbon ion or proton therapies for tissue ablation. Examples of conditions which may benefit from BNCT include non-malignant diseases, non-metastatic benign tumours, uterine fibroids, arthritis, breast adenoma, menohrragina, benign prostate hyperplasia and destruction of selective cardiac structures or clots. BNCT may also be used where tissue ablation is preferable to procedures such as surgery, photodynamic therapy, cryosurgery, thermal laser ablation and vaporisation.

All preferred features of the first, second, third, fourth, fifth and sixth aspects of the invention also apply to the seventh aspect.

The eighth aspect of the invention relates to the use of a composition of the first or third aspects or a formulation of the fifth aspect in the manufacture of a medicament for use in the treatment of cancer, specifically in boron neutron capture therapy.

All preferred features of the first, second, third, fourth, fifth, sixth and seventh aspects of the invention also apply to the eighth aspect.

The ninth aspect provides a method of treating cancer comprising administering the composition of the first or third aspects or the formulation of the fifth aspect to a patient and then subjecting the patient to neutrons or radiotherapy or other ionising or non-ionising photons, or atomic or subatomic particle therapies such as photons, carbon ion or proton therapies. The eighth aspect provides a method for treating cancer of the breast, lung, head, neck, bone, prostate, pancreas, cervix, skin and lymphomas.

There is supplied to the recipient of the composition a supply of neutrons, which react with the boron atoms of the composition to produce an alpha particle which is capable of ablating the cell. The neutron supply is preferably in the form of monochromatic neutrons, which are free from contaminants such as fast neutrons, and gamma, beta radiation or X-rays. The use of clean neutrons free from such contaminants ensures a superior biological outcome compared to the results obtained with conventional, contaminated neutron beams. The monochromatic neutrons used in the present invention may be obtained from various sources, such as the fusionstar device of Sved *et al* (Am. Inst. Phys 1-56396-825/99 (1999) the contents of which are incorporated herein by reference, and available from Daimler-Chrysler Aerospace). Preferably, the neutrons emitted from these sources have an energy level of between 0.01eV and 0.5eV, and most preferably 0.1eV and 0.01eV.

The neutrons are preferably supplied as a beam, which has a cross section larger that the tissue to be ablated. Typically, the beam will have a cross section such that it is capable of capturing tissue slightly outside the tissue to be ablated, i.e. having a margin of between 0.5mm to 10mm. The beam is preferably adjusted according to the size and location of the tissue to be ablated, and the exact cross section of the beam may be calculated according to

these variables by persons skilled in the art. In practice, after administration of the composition, sufficient time is allowed to elapse to enable clearance of the composition from normal tissue, in order to minimise the damage to such tissue. Clearance times will of course depend on various factors and can be easily calculated by persons skilled in the art. At the appropriate time, the patient is positioned in front of the neutron beam, so that the target tissue is within the neutron irradiation field. The physical parameters of the beam and the boron distribution within the patient will ensure that the radiation exposure of the target tissue is maximised, and that of the normal or healthy tissue is minimised.

All preferred features of the first, second, third, fourth, fifth, sixth, seventh and eighth aspects of the invention also apply to the ninth aspect.

The invention is illustrated by reference to the following figure:

Figure 1 shows the boron concentrations in blood and in the tumour as a function of time after the injection of boronophenylalanine in two formulations of the invention.

The present invention will now be illustrated by reference to the following non-limiting examples.

Examples:

Example 1

200mg of BPA is mixed with 202mg lactobionic acid and brought to pH 10 with 2.5M sodium hydroxide solution (0.45ml). The final pH is adjusted to 8.9 - 9.0 with a 2M solution of hydrochloric acid. The final volume is adjusted to 1.0ml with saline giving a 200mg / ml solution that is suitable for intravenous injection.

The solution remains clear and stable for at least 9 to 12 months.

Example 2

200mg of BPA is mixed with 200mg of D-gluconic acid sodium salt and brought to a pH of 9.5, with a solution of 2.5M sodium hydroxide in water (0.75ml). The final pH is adjusted to 8.8 - 8.9 with a 2M solution of hydrochloric acid. The final volume is adjusted to 1.0ml with distilled water.

The solution remains clear and stable for at least 9 to 12 months.

Example 3

200mg of BPA is mixed with 200mg of D-gluconic acid sodium salt and brought to a pH of 9.5, with a solution of 2.5M sodium hydroxide in water (0.75ml). The final pH is adjusted to 8.8 - 8.9 with a 2M solution of hydrochloric acid. 20mg of fructose (0.11mole) is added. The final volume is adjusted to 1.0ml with distilled water.

The solution remains clear and stable for at least 9 to 12 months.

Example 4

100mg of BPA is mixed with 150mg tri-sodium citrate and brought to pH 9.5 with the addition of 2.5M sodium hydroxide solution. The final pH and volume are adjusted to 8.8 - 8.9 and 1.0ml as previously described.

The final solution remains stable and clear for at least 3 months.

Example 5

100mg of BPA is mixed with 25mg tri-sodium citrate and 50mg of Solutol HS (BASF) (Polyethylene glycol 12-hydroxystearate) and brought to pH 9.5 with the addition of 2.5M sodium hydroxide solution. The final pH and volume are adjusted to 8.8 - 8.9 and 1.0ml as previously described.

Solution [5] remained clear and stable for at least 3 months.

Example 6

100mg of BPA is mixed with 25mg tri-sodium citrate and 50mg of Tween 80 (polyoxyethylene sorbitan mono laurate) and brought to pH 9.5 with the addition of 2.5M sodium hydroxide solution. The final pH and volume are adjusted to 8.8 - 8.9 and 1.0ml as previously described.

Solution [6] remained clear and stable for at least 3 months.

Example 7

Formulation 1: Boronophenyalanine (BPA) was dissolved in gluconic acid. The pH was then adjusted to 8.0 by the addition of caustic soda (NaOH). The 200 mg/ml stock solution was diluted to 150 mg/ml by the addition of normal saline.

Formulation 2: Boronophenyalanine (BPA) was dissolved in lactobionic acid. The pH was then adjusted to 8.0 by the addition of caustic soda (NaOH). The 200 mg/ml stock solution was diluted to 150 mg/ml by the addition of normal saline.

Administration protocol

Male Fisher 344 rats aged 12 weeks (256-301g) at the commencement of experimental procedures were used. Rats were housed two to a cage in temperature-controlled rooms and had free access to food and water. Rats were maintained in a controlled light/dark cycle, with lights on between 0700 and 1900h. The animal studies were reviewed and approved by the Resident Institutional Animal Care and Use Committee.

p-Boronophenylalanine (BPA; L-enantiomer, > 98% ^{10}B-enriched; Ryscor, Inc., Raleigh, NC) containing 4.9% boron by weight was used as the boron delivery agent. BPA was administered in formulations F1 and F2 detailed above that contained approximately 29 mg boron/ml. Administration was by i.p. injection, at a volume of 1 ml that delivered approximately 600 mg BPA/kg.

Boron analysis

Concentrations of boron in BPA solutions or in tissue samples were determined using direct current plasma atomic emission spectroscopy (DCP-AES).The biodistribution data for tumour and normal tissues is detailed in Table 1 below.

Tumour inoculations

Rats were inoculated both intracranially (10^4 cells/ 1µl) and subcutaneously (10^6 cells/ 0.1ml) with 9L gliosarcomas tumour cells.

The rat GS-9L gliosarcoma cell line was maintained in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum (inactivated). Tumour inoculation into the brain was carried out by injection of 1 x 10^4 cultured cells in 1 µl of medium into the left frontal lobe of rats weighing ~200 g. Under aseptic conditions, the scalp of the anaesthetised rat was incised and a 0.5 mm bur hole made in the skull, at a point 4 mm to the left of the midline and directly on the bregma. Tumour cells were delivered at a depth of 5 mm in the brain using a 27-gauge needle fitted with a depth-limiting plastic collar.

Biodistribution

Tumour uptake of BPA was comparable using both formulations, as was the biodistribution of the compound in normal tissues (Table 1). There was no appreciable difference in the uptake of BPA in i.c. or s.c. implanted tumours. Considered overall the results were comparable with those obtained using BPA in the conventional fructose formulation at similar doses (Figure 1). Visual observation of animal appearance, levels of activity and general behaviour indicated no evidence of toxicity using formulations F1 and F2.

Table 1. Boron concentrations in blood, rat 9L gliosarcoma tumour, brain, skin and liver as a function of time after i.p. injection of ~ 600 mg/kg of BPA dissolved in formulation F1 or F2 (± S.E.).

Time after injection (h)	Blood (μg/g)	Tumour (μg/g)	Brain (μg/g)	Skin (μg/g)	Liver (μg/g)
Formulation 1					
1	23.5 ± 0.7	45.2 ± 1.5 s	-	-	-
3	14.9 ± 0.2	44.2 ± 2.0 s 39.7 ± 4.9 i	10.1 ± 0.6	15.9 ± 1.7	14.5 ± 0.4
6	9.7 ± 0.3	28.1 ± 0.9 s 32.9 ± 1.3 i	7.9 ± 0.5	15.1 ± 0.4	9.7 ± 0.4
10	7.1 ± 0.3	22.0 ± 0.4 s 23.5 ± 2.2 i	7.5 ± 0.5	7.2 ± 0.2	7.6 ± 0.3
Formulation 2					
1	25.5 ± 1.8	48.0 ± 1.9s	-	-	-
3	14.0 ± 1.0	46.5 ± 1.7 s 45.8 ± 4.7 i	10.2 ± 0.5	17.8 ± 3.0	14.2 ± 0.4
6	10.5 ± 0.2	30.2 ± 1.4 s 28.3 ± 4.6 i	9.1 ± 0.3	12.8 ± 2.6	10.2 ± 0.2
10	7.2 ± 0.1	20.8 ± 1.1 s 24.2 ± 2.2 i	6.5 ± 0.2	7.4 ± 0.1	7.3 ± 0.1

i = intracranial: s = subcutaneous

Conclusions

The formulations 1 and 2 delivered similar biodistribution profiles to those obtained using boronophenylalanine in the conventional fructose formulation at similar doses. However, the concentrations of boronophenylalanine were a factor of 3 higher than the highest deliverable concentration of boronophenylalanine in the conventional fructose formulation. Higher concentrations of boronophenylalanine (factor of 4) could be given if required.

The tumour:blood boron concentration ratio was ~3:1 using both formulations

From a clinical perspective, much lower infusion volumes could be administered to patients using the new formulations of boronophenylalanine.

Particulate compositions

Example 1 Preparation of nanoparticles

BPA, CuTCPH and CuTCPBr were used to prepare particulate suspensions. BPA in the from of a powder 100μ average particle size from Kathchem was used. Polyvinylpyrrolidone K15 , Molecular weight~10000, from Aldrich, Product code 81390 was used to condition the surface of the particles. The mills were operated for 24-48 hours

Two mills were used to generate particulate suspensions as follows;

Conventional Mill 1

A Porcelain mill with an internal diameter of 110 mm and a depth of 100 mm operated at a rotation speed 1.25 Hz (35% of critical speed) with porelain millig media in the form of 10 mm diameter spheres that occupies a total volume of ~500ml.

E.g.Mixture volume

BPA	7.5 g
PVP	3.0 g
Water	~200 ml

Customised Mill 2

This mill was designed design not to surge and be good for mixing and attrition which is the most effective mechanism for fine grinding and produces a uniform particle size.

The stainless steel mill of internal diameter 50 mm and depth of 50 mm was operated at a rotation speed of 1.25 Hz (14% of critical speed) with a milling media composed of 3mm diameter stainless steel spheres (2000 off) occupying a volume ~50 ml.

Eg. Mixture volume (max)	~50
BPA	2.50 g
PVP	0.75 g
Water	50 ml

Over 75% of the particles are below 400nm. These particles can be further separated out using filters of particular pore size. A solution of 5mg/ml containing 200nm particles is produced by filtration through a 200nm pore filter. This solution is simply concentrated up to 20 mg/ml CuTCPBr by evaporating off the water at 60°C (remains stable, clear, does not precipitate or flocculate, concentrated formulations can be produced up to 200mg/ml). This solution was injected into tumour bearing mice as described in Example 7. The compound accumulates in the tumour mass with a ratio of 4:1 at 24 hours. Blood to liver ratios are comparatively low (1:2) at 24 hours. This indicates that the particles are evading the endoreticulo system (liver, spleen) and that the stealth layers are working. This demonstrates that nano particulate formulations can be used for therapy.

CLAIMS

1 A composition comprising a boron-containing compound and a polyhydroxy acid in a solution having a pH of 7.5 to 10.5.

2 A composition as claimed in claim 1 wherein the boron-containing compound is boronophenylalanine.

3 A composition as claimed in claims 1 or 2 further comprising fructose.

4 A composition as claimed in any one of claims 1 to 3 further comprising an emulsifier.

5 A composition as claimed in any one of claims 1 to 4 wherein the boronophenylalanine is provided at a level of from 100 to 250mg per ml.

6 A composition as claimed in any one of claim 1 to 5 wherein the polyhydroxy acid is one or more of lactic acid, citric acid, glucaric acid, gluconic acid, lactobionic acid, erythronic acid, glycerophosphate acid, mannuronic acid or sorbic acid.

7 A composition as claimed in any one of claims 1 to 6 wherein the emulsifier is one or more of gelatin, casein, lecithin (phosphatides), gum acacia, calcium stearate, cholesterol, tragacanth, sorbitan esters, stearic acid, benzalkonium chloride, glyceryl monostearate, cetostearl alcohol, cetomacrogol emulsifying wax, , polyoxyethylene alkyl ether, macrogol ethers, cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene

glycols, polyoxyethylene stearates, phosphates, sodium dodecylsulphate, bile acids, deoxycholic acid , cholic acid chenodeoxycholic acid, tauric acid, triethanolamine, polyvinyl alcohol (PVA), and polyvinypyrrolidonr (PVP), block copolymers of ethylene oxide and propylene oxide; polyvinyl pyrrolidone, Pluronic F68, Pluronic F108, Tetronic 908, tetrafuctional block copolymer derived from the addition of ethylene oxide and propylene oxide to ethylenediamine, dextran, lecithin, polyethylene glycols, dioctyl ester of sodium sulfosuccinic acid, polyoxyethylene sorbitan fatty acid ester, sodium lauryl sulfate, alkylaryl polyether sulfonate, lecithin, Pluronic F-68, polyvinylpyrrolidone, polyoxyethylene 20 sorbitan monolaurate, polyoxyethylene 20 sorbitan monopalmitate, polyoxyethylene 20 sorbitan monostearate and polyoxyethylene 20 sorbitan monooleate or a mixture of two or more thereof.

8 A process for the production of a composition as claimed in any one of claims 1 to 7 comprising mixing boronophenylalanine with a polyhydroxy acid in a solution and adjusting the solution to a pH of 7.5 to 10.5.

9 A composition comprising a stable particulate suspension of a boron containing compound and a surfactant.

10 A composition as claimed in claim 9 wherein the boron containing compound is one or more of BPA, CuTCPH or CuTCPBr.

11 A composition comprising a composition as claimed in claims 1 to 7 in combination with a composition as claimed in claims 9 or 10.

12 A process for the production of a composition as claimed in claims 9 or 10 comprising grinding the boron containing compositions to form a powder and contacting the particles formed with a surfactant.

13 A process for the production of a composition as claimed in claim 11 comprising mixing a composition as claimed in any one of claims 1 to 7 with a composition as claimed in claims 9 or 10.

14 A composition as claimed in any one of claims 1 to 7 or any one of claims 8 to 11 for use medicine.

15 A composition as claimed in claim 14 for use in the treatment of cancer.

16 A composition as claimed in claim 14 or claim 15 for use in boron neutron capture therapy.

17 The use of a composition as claimed in claims 1 to 7 or in claims 9 to 10 in the manufacture of a medicament for use in the treatment of cancer.

18 The use as claimed in claim 17 in boron neutron capture therapy.

19 A method for the treatment of cancer comprising the steps of administering a composition as claimed in claims 1 to 7 or 8 to 10, followed by administering a supply of neutrons.

20 A method as claimed in claim 19 wherein the supply of neutrons is in the form of monochromatic neutrons.

21 A method as claimed in claims 19 or 20 wherein the neutrons have an energy range of 0.01eV to 0.5eV.

Figure 1

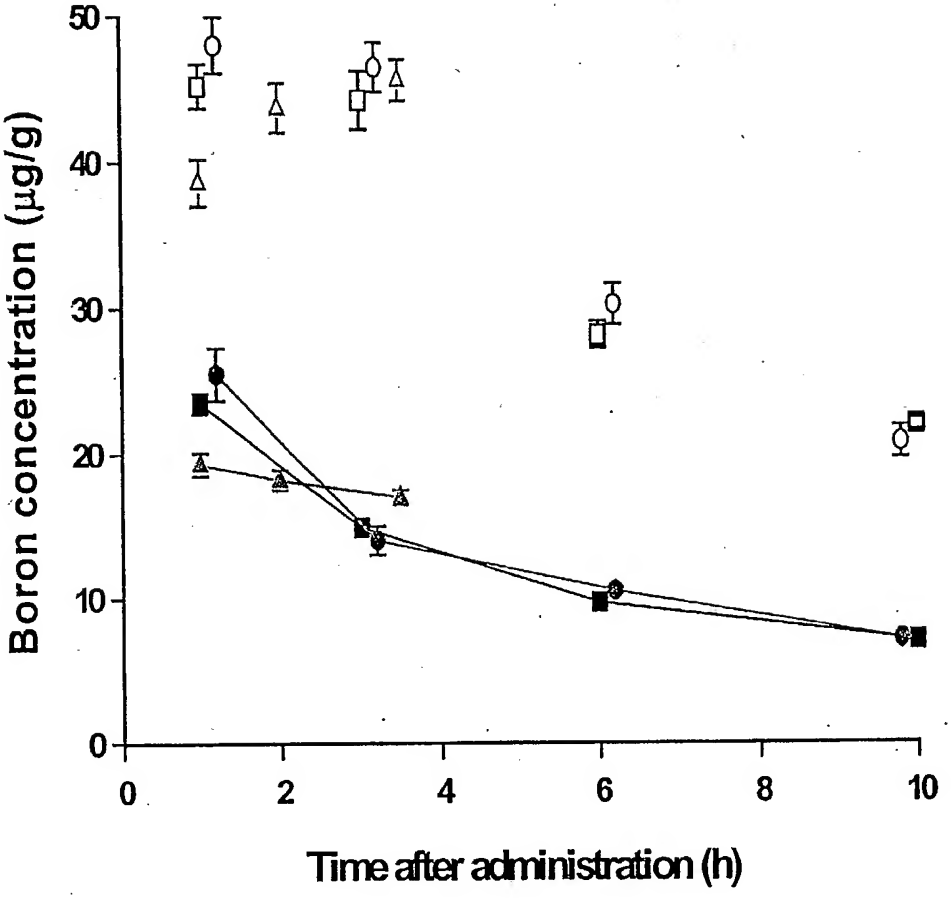


Figure 1. Boron concentrations in blood and tumour as a function of time after i.p. injection of ~ 600 mg/kg of BPA dissolved in formulation F1 (■) or F2 (●). By way of comparison previously obtained data (▲) using ~ 650 mg/kg of BPA dissolved in fructose is shown. Solid symbols represent blood and open symbols represent tumour. Error bars that are not visible are contained within the symbol (± S.E.).